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Significance of Cytomegalovirus (CMV)-pp65 Antigenemia in the Diagnosis of CMV Disease in Human Immunodeficiency Virus-Infected Patients

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To establish the diagnostic value of the cytomegalovirus (CMV)-pp65 antigenemia in CMV disease occurring in human immunodeficiency (HIV)-infected patients, CMV-pp65 antigen in polymorphonuclear leukocytes (PMNLs) was assayed in 373 samples from 138 randomly included patients followed up for symptomatic HIV-1 infection and the correlation between CMV-pp65 antigenemia and diagnosis of CMV disease was investigated. Thirty-seven CMV disease episodes were observed in 30 patients and 89.2% of these episodes were associated with a positive CMV-pp65 antigenemia. In contrast, 94% of the patients negative for CMV-pp65 antigenemia remained free of CMV disease. Patients with CMV disease had significantly higher levels of CMV-pp65 antigenemia than CMV disease-free patients (695 positive cells/2 $\times$ 10^5 PMNLs vs. 28 positive cells/2 $\times$ 10^5 PMNLs). The positive and negative predictive values of the test were 45% and 94%, respectively, but were 93% and 80%, respectively, when a CMV-pp65 antigenemia level of >100 positive cells/2 $\times$ 10^5 PMNLs was taken into consideration. These results indicate that the CMV-pp65 antigenemia assay is useful for the diagnosis and monitoring of CMV disease in HIV-infected patients.

KEY WORDS: AIDS, retinitis, colitis, encephalitis, monitoring, predictive value

INTRODUCTION

Cytomegalovirus (CMV) represents a major cause of diseases such as retinitis, esophagitis, colitis, or encephalitis in patients with acquired immunodeficiency syndrome (AIDS) [Jacobson and Mills, 1988; Drew, 1992]. CMV viremia is observed frequently in these patients [Quinnan et al., 1984; Fiala et al., 1986; Segondy et al., 1990], and it has been reported that a CMV disease occurs in half of the patients who develop CMV viremia after a mean delay of more than 7 months [Salmon et al., 1990]. Another study, which evaluated the clinical use of CMV cultures in human immunodeficiency virus (HIV)-infected patients, showed that positive CMV-blood cultures are poorly predictive of the subsequent development of a CMV disease and have limited diagnostic interest [Zurlo et al., 1993].

A diagnostic assay that detects CMV antigen in peripheral blood polymorphonuclear leukocytes (PMNLs) has been developed [van der Bij et al., 1988b; Gerna et al., 1990]; the antigen detected in the PMNLs has been identified as the pp65 lower matrix phosphoprotein of the CMV [Revello et al., 1992]. The CMV-pp65 antigenemia assay, which is more sensitive than CMV culture for the detection of CMV in blood [van der Bij et al., 1988a; Erice et al., 1992], allows the quantitation of pp65 antigen-positive PMNLs [van der Bij et al., 1988a; Gerna et al., 1990]. It has been found that high CMV-pp65 antigenemia levels are correlated with CMV diseases in transplant recipients [van den Berg et al., 1989, 1991; Gerna et al., 1990; Mazzulli et al., 1993] and in patients with AIDS [Mazzulli et al., 1993].

In the present study, the clinical use of the CMV-pp65 antigenemia assay was evaluated in HIV-1-infected patients and the value of the test for the diagnosis of CMV diseases was determined.

MATERIALS AND METHODS

Patients

Between February and December 1994, 373 blood samples were obtained from 138 randomly included adult patients (117 men and 21 women) followed up for symptomatic HIV-1 infection in the AIDS Day Care Unit in the Department of Infectious and Tropical Diseases of our hospital. The age was from 20 to 60 years (mean: 35.8 $\pm$ 7.9 years). According to the classification of the Centers for Disease Control and Prevention [1993], 39

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patients were classified as category B (B1: 4, B2:9, and B3: 26) and 99 were classified as category C (C2: 7, C3: 92); they had a CD4+ cell count of (77 ± 245) × 10^6/l (median: 20 × 10^6/l; range: 1–764 × 10^6/l). The patients were seen monthly and followed up during at least 4 months after the last determination of CMV-pp65 antigenemia.

Diagnostic Criteria

CMV retinitis was diagnosed by indirect ophthalmoscopy through dilated pupils; diagnosis was based on the observation of typical retinal opacity with perivascular distribution and perivascular hemorrhages. Diagnosis of CMV gastrointestinal disease was established in patients with gastrointestinal symptoms by the presence of typical lesions at esophagogastroduodenoscopy or coloscopy and the isolation of CMV associated with the presence of CMV inclusions in biopsy specimens. Diagnosis of CMV encephalitis in patients with central neurologic symptoms and compatible tomodensitometric signs was established by the detection of CMV DNA in cerebrospinal fluid by the polymerase chain reaction [Gozlan et al., 1992]. Diagnosis of CMV pneumonitis was established in a patient with respiratory signs by the isolation of CMV in the bronchoalveolar lavage (BAL) associated with the observation of CMV-specific cytopathic effects in the BAL cells in the absence of other pathogens.

CMV-pp65 Antigenemia Assay

The CMV-pp65 antigenemia assay was carried out on 373 specimens according to the optimized procedure described by Gerna et al. [1992]. The CMV-pp65 antigen was detected by using the IgG monoclonal antibody 1C3 (Argène, Varilhes, France) which has been evaluated in previous reports [Gerna et al., 1992; Revello et al., 1992]. Briefly, PMNLs were isolated from 5 ml of freshly collected heparinized blood by sedimentation for 20 min at 37°C in the presence of 1 ml of a 6% dextran solution. PMNLs were recovered by centrifugation (200*g*) for 10 min at room temperature and resuspended at a concentration of 2 × 10^6/ml. After lysis of the few remaining erythrocytes by a 0.8% NH_4Cl solution, 2 × 10^5 PMNLs (100 μl) were cytocentrifuged onto glass slides. Cytospin preparations were fixed for 10 min at room temperature with 5% formaldehyde-2% sucrose in phosphate-buffered saline (PBS) and permeabilized for 5 min with 0.5% Nonidet P-40-10% sucrose-1% fetal calf serum in PBS. After drying, CMV-pp65 antigen was detected by indirect immunofluorescence by using the monoclonal antibody 1C3 diluted 1:20 and a fluorescein-conjugated goat anti-mouse IgG (Argène) diluted 1:200. Positive cells were counted under an ultraviolet (UV) microscope at a ×400 magnification. Results were expressed as positive cells per 2 × 10^5 PMNLs examined. For the highest CMV-pp65 antigenemia levels (>500 positive cells/2 × 10^5 PMNLs), the positive cells were counted in 6 different fields and the number was multiplied by 10 to account for the whole cytospin preparation.

TABLE I. Relationship Between the Results of the CMV-pp65 Antigenemia Assay and CD4+ Cell Count for HIV-1-Infected Patients With or Without CMV Disease

CD4+ cell count (× 10^6/l)	CMV-pp65 antigenemia assay (No. of patients)			
	Positive		Negative	
	Disease	No disease	Disease	No disease
1–9	17	14	3	19
10–49	8	12	1	19
50–99	1	5	0	16
100–199	0	2	0	5
≥200	0	1	0	15

Statistical Analysis

Results are expressed as mean ± SD. The χ^2 test was used to compare differences in the frequency of positive CMV-pp65 antigenemia between groups, and Student's t-test for comparison of means.

RESULTS

Among the 138 patients with symptomatic HIV-1 infection included in this study, 30 (22%) presented 37 episodes of CMV disease involving 39 organs: 28 retinitis, 6 colitis, 3 encephalitis, 1 esophagitis, and 1 pneumonitis; retinitis was associated with encephalitis in 1 patient, and with colitis and encephalitis in another patient. Among the 373 CMV-pp65 antigenemia assays undertaken, 151 (40.5%) were positive for 60 (43.5%) patients. Thirty-three (89.2%) CMV disease episodes occurring in 26 patients were associated with a positive CMV-pp65 antigenemia assay and 4 (3 retinitis, 1 colitis) in 4 patients were not ($P < 0.001$). For 10 patients followed up for more than 6 months who developed a positive CMV-pp65 antigenemia and presented a subsequent progression toward a CMV disease, the delay between the positivity of the assay and the diagnosis of the disease was 102 ± 39 days (range: 40–172 days).

The CD4+ cell count was (95 ± 150) × 10^6/l (median: 42 × 10^6/l; range: 1–764 × 10^6/l) for the patients with negative CMV-pp65 antigenemia and (29 ± 42) × 10^6/l (median: 13 × 10^6/l; range: 1–330 × 10^6/l) for the patients with positive CMV-pp65 antigenemia assay ($P < 0.001$). As shown in Table I, all the CMV diseases were observed in patients with a CD4+ cell count of <100 × 10^6/l and all but one of these patients had a CD4+ cell count of <50 × 10^6/l ($P < 0.001$). The positivity of the CMV-pp65 antigenemia assay was also associated significantly with a CD4+ cell count of <50 × 10^6/l ($P < 0.001$).

In patients who had CMV-pp65 antigenemia during follow-up, the antigenemia level was 28 ± 45 positive cells/2 × 10^5 PMNLs (median: 12 positive cells/2 × 10^5 PMNLs; range: 1–240 positive cells/2 × 10^5 PMNLs) for patients without CMV diseases and 695 ± 1,219 positive cells/2 × 10^5 PMNLs (median: 61 positive cells/2 × 10^5 PMNLs; range: 1–5,000 positive cells/2 × 10^5 PMNLs) for patients with CMV disease ($P < 0.01$). The positive

TABLE II. Positive and Negative Predictive Values of Several Levels of CMV-pp 65 Antigenemia for the Diagnosis of CMV Disease

Antigenemia (cells/2 × 10^5 PMNLs)	Positive predictive value (%)	Negative predictive value (%)
>0	45	94
>10	49	86
>50	78	80
>100	93	80
>500	100	77
>1,000	100	74

and negative predictive values for the diagnosis of CMV disease calculated for different CMV-pp65 antigenemia levels are shown in Table II.

DISCUSSION

The diagnostic value of the CMV-pp65 antigenemia assay was evaluated in CMV disease occurring in HIV-infected patients. CMV disease was observed in 26% of the patients who had a CD4+ cell count of $<100 \times 10^6$/l; retinitis represented 76% of the diseases. These frequencies are similar to those reported by others [Jacobson and Mills, 1988; Drew 1992]. Our results showed that the CMV-pp65 antigenemia assay is of clinical value for diagnosis of CMV disease in HIV-infected patients. Indeed, 94% of the patients without CMV-pp65 antigenemia were free of CMV disease, whereas 45% of the positive patients developed a CMV disease. We also observed that patients who became positive for CMV-pp65 antigenemia during the course of the follow-up developed a CMV disease after a mean lapse of time of approximately 3 months. Furthermore, when the antigenemia level was considered it was observed that the positive predictive value of the test increased to >90% when the antigenemia was >100 cells/2 × 10^5 PMNLs and reached 100% when antigenemia was >500 cells/2 × 10^5 PMNLs.

It has been reported that the CMV-pp65 antigenemia assay may be a tool for the diagnosis and monitoring of CMV disease in solid organ or bone-marrow transplant recipients [van den Berg et al., 1989; Gondo et al., 1993]; a level of >50 positive cells/2 × 10^5 PMNLs has been considered indicative of CMV disease in these patients [van den Berg et al., 1989; Gerna et al., 1991]. High levels of CMV-pp65 antigenemia have also been noted in AIDS patients with CMV disease [Gerna et al., 1990; Mazzulli et al., 1993]. The results obtained in the present study are in accord with these reports.

Considering the high negative predictive value of the CMV-pp65 antigenemia, the assay could be useful in HIV-infected patients with a CD4+ cell count of $<100 \times 10^6$/l for identifying those at risk of CMV disease. High levels of CMV-pp65 antigenemia (>100 positive cells/2 × 10^5 PMNLs) are associated significantly with CMV organ involvements; therefore, the CMV-pp65 antigenemia assay might be used for monitoring patients at risk and we suggest that a rise in the antigenemia level above 100 positive cells/2 × 10^5 PMNLs, mainly in patients with a CD4+ cell count of $<50 \times 10^6$/l, should be considered a criterion for initiating an anti-CMV therapy, even in the absence of clinical signs of CMV disease.

Quantitative polymerase chain reaction (PCR) is an alternative method for the determination of the CMV load in peripheral blood cells [Zipeto et al., 1993; Drouet et al., 1995]. A study conducted in HIV-infected patients showed that subjects with a CD4+ cell count of $<100 \times 10^6$/l who had CMV retinitis significantly presented a higher copy number of CMV DNA in their peripheral blood cells than did either patients free of CMV retinitis or patients with a CD4+ cell count of $>100 \times 10^6$/l. Therefore, a high copy number of CMV DNA could predict CMV retinitis in patients with a CD4+ cell count of $<100 \times 10^6$/l and it has been suggested that monitoring for increases in CMV DNA copy number might allow identification of HIV-infected patients at imminent risk for the development of CMV retinitis [Rasmussen et al., 1995]. The results obtained in the present study also lead to the conclusion that an increase in CMV load in peripheral blood cells is predictive of CMV disease in HIV-infected patients. Quantitative PCR is, however, an expensive and time-consuming technique which has not been standardized yet for CMV DNA quantitation. For these reasons, we suggest that the CMV-pp65 antigenemia assay may be, at present, a more appropriate technique for monitoring HIV-infected patients at risk of CMV disease, because it is rapid (4 hr), less expensive, and available commercially.

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